STUDIES OF SULFUR METABOLISM

THE OXIDATION OF SOME SULFUR COMPOUNDS RELATED TO CYSTINE IN THE ANIMAL ORGANISM

BY

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THE METABOLISM OF SULFUR.

VII. THE OXIDATION OF SOME SULFUR COMPOUNDS RELATED TO CYSTINE IN THE ANIMAL ORGANISM.*

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In previous studies (1, 2, 3) one of us (L.) has reported experiments which are concerned with the oxidation of sulfur in the cystine molecule. It was demonstrated that if deamination of cystine be prevented by "blocking" the amino group (as in phenyluraminocystine (1) or dibenzoylcystine (3)), the sulfur of the molecule was not oxidized normally and was excreted in the urine in large part in the unoxidized sulfur fraction. However, if normal oxidation of the sulfur was prevented in this way, it was shown that the cystine was converted to cysteine and a considerable amount of the cystine derivative administered appeared in the urine as a derivative of cysteine (2, 3). Recently Sherwin and his collaborators (4, 5) have reported similar results which in general confirm our earlier experiments. They also showed that if a derivative of cysteine with the amino group protected from deamination was fed to a rabbit, the cysteine was in part converted to cystine. Our own results and those of Sherwin are in accord with the current theory of the ready reversibility of the reaction cysteine $\rightleftarrows$ cystine.

Many other investigations which are related to the oxidation of various types of sulfur linkages in organic combination have been reported. Inasmuch as this present study is concerned only with the oxidation of mercapto or sulfide sulfur of the type similar to that naturally occurring in the protein molecule, a complete

* An abstract of a thesis submitted by R. M. Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Illinois.

review of the literature on the oxidation of organic sulfur compounds is not attempted. The discussion of the oxidation of cyclic compounds containing sulfur in the ring (*e.g.*, thiophene, thioindoxyl) is omitted entirely and will be discussed in another place by one of us (L.).

Investigators who have studied the oxidation of the sulfur of the mercapto groups in aliphatic compounds are generally agreed that sulfur of this type is oxidized to sulfates and excreted in the urine in this form to a considerable degree. The ready oxidation of the sulfur of cysteine is well known. Smith (6) fed small doses of the sodium salt of ethyl mercaptan to dogs and noted slight rises in the sulfate sulfur of the urine. In one experiment in which a total of about 1.6 gm. was fed to a 7.5 kilo dog over a period of 4 days, 55 per cent of the sulfur fed appeared as extra sulfur in the urine and of this 53.7 per cent was present as sulfate sulfur. In a second similar experiment in which 4.99 gm. were fed during a 5 day period, 47 per cent of the ingested sulfur appeared in the urine, and 37.5 per cent of this extra sulfur was present as sulfate sulfur. Ethyl mercaptan itself was somewhat more toxic than the sodium salt. Of 1.021 gm. fed in a single dose, slightly less than 25 per cent was eliminated in the urine, and of this 70 per cent was sulfate sulfur. The interpretation of these experiments is complicated by the fact that the mercaptan was always somewhat toxic and that an increased nitrogen elimination was usually noted. Smith (7) also reports one experiment in which 2 gm. of the ammonium salt of thioglycollic acid were fed daily to a dog for 2 days. A larger dose caused vomiting and other toxic symptoms. The sulfate sulfur excretion was somewhat increased, indicating a partial oxidation of the sulfur of the complex. Smith (8) also fed ethyl thiolcarbamate (thiourethane), $NH_2CO \cdot S \cdot C_2H_5$, and ethyl thiocarbamate, $NH_2CS \cdot O \cdot C_2H_5$, to dogs. The latter was much more toxic than the former and little oxidation (?) of its sulfur was noted. The sulfur of ethyl thiolcarbamate, however, was oxidized to sulfates to a considerable extent. It is possible that in this case hydrolysis to ethyl mercaptan may have occurred with subsequent oxidation of the latter. Freise (9) noted after the enteral administration of thiouramil to dogs that the sulfur of its mercapto group was partially (48.1 per cent of the total sulfur recovered in

the urine) oxidized, while after feeding γ-thiopseudouric acid, although only 36 per cent of the sulfur fed was eliminated as urinary sulfur, of this 86 per cent appeared in the sulfate sulfur fraction.

The oxidation of the sulfur of compounds containing the disulfide sulfur linkage, -S-S-, has not been studied except in the case of cystine. In his study, Smith (10) has noted that the sulfur of ethyl sulfide was eliminated very slowly and that no evidence of any oxidation was apparent. Bivalent sulfur, which replaces oxygen in carbonyl groups, is apparently not oxidized as illustrated in the case of thiourea (11), the thiohydantoins (12), and thiopyrimidines (13). The partially oxidized sulfur of sulfonic acids and sulfones is resistant to further oxidation (7, 14).

Thus there are recorded studies of the behavior of compounds containing sulfur in the types of linkage, C-SH (mercaptan), C-S-C (sulfide), C-SO_2. OH (sulfonic acid), C-SO_2-C (sulfone), = C = S (thioamide), and C-S-S-C (disulfide). Of these only the first, C-SH, and such others as may be hydrolyzed (*e.g.* thiourethane) or reduced (cystine) to form this type are readily oxidized in the organism. This is the more remarkable, in view of the fact that some of the other more highly oxidized forms are intermediary products of the oxidation of the mercaptan group *in vitro*. The mercaptan group, less readily oxidized *in vitro*, is the most readily oxidized *in vivo*.

In the present investigation, we have undertaken a study of the oxidation of some sulfur compounds which have a closer relationship to cystine or cysteine than most of those previously studied in order to obtain a more complete picture of the conditions governing oxidation of sulfur in the animal body.

EXPERIMENTAL.

The rabbits used in the experiments were fed daily 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay, with water *ad libitum*. The sucrose was added to increase the calorific value of the diet and the hay in order to provide roughage, since we have found repeatedly that animals on such a concentrated diet as milk and sugar are better nourished if a small amount of hay be added also. This may be a question of roughage or of some other factor. This standard diet was fed for at least 3 days before the collection of

the urine was begun in order to be certain that the animal was accustomed to the diet and to insure as uniform a urinary excretion as possible. The urine was then collected in 24 hour periods by squeezing out the bladder and samples for at least 3 normal days were obtained and analyzed. The substance under investigation was fed through a stomach tube or injected subcutaneously and changes in the distribution of sulfur in the urine were noted. An after period completed each experiment.

Total sulfur was determined by the Denis modification of Benedict's method, inorganic sulfate sulfur and total sulfate sulfur by the methods of Folin, conjugated sulfate sulfur and organic sulfur were obtained by difference, as is customary. Nitrogen determinations by the usual Kjeldahl method were carried out on all samples in order to afford a check on any changes in protein metabolism. An increase in protein metabolism or tissue breakdown due to the toxic action of the substance administered would increase the sulfur elimination and might make it appear that an oxidation of the substance under investigation had occurred, when this had not been the case. If an increase in the sulfur output were due to increased catabolism of protein, this should be accompanied by a corresponding increase in the output of total nitrogen.

The compounds under investigation, thioglycollic, thiolactic, and thiodiglycollic acids, were Kahlbaum preparations, the purity of which was checked by analysis of the sulfur content.

DISCUSSION.

Thiolactic Acid, $CH_3 \cdot CH(SH) \cdot COOH$.—The results of typical experiments[1] with thiolactic acid are detailed in Tables I and II. The animals remained bright, there was no loss of appetite, and no toxic effects were noted at any time. In the experiment recorded in Table I, approximately 90 per cent of the sulfur fed as thiolactic acid was eliminated as "extra" sulfur the 1st day,

[1] In the experiments with thiolactic acid as well as those with the other compounds studied, a few typical experiments only are reported. In all cases, these results were checked with other animals. In order to condense the data these additional experiments are omitted. They are, however, recorded in the original thesis on file in the Library of the University of Illinois.

and 56 per cent of this appeared as extra inorganic sulfate. In the two experiments of Table II, 78 per cent (oral administration) and 84 per cent (subcutaneous injection), respectively, of the sulfur administered, was eliminated as extra sulfur the 1st day and of this 71 and 61 per cent, respectively, were completely oxidized,

TABLE I.

Rabbit I. Male. Weight 2.5 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0233	0.0151	0.0019	0.0063	0.589	
2	0.0242	0.0130	0.0049	0.0063	0.593	
3	0.0229	0.0102	0.0048	0.0079	0.603	0.658 gm. thiolactic acid as sodium salt *per os*. (S=0.1974 gm.)
4	**0.1986**	**0.1137**	**0.0088**	**0.0761**	**0.582**	
5	0.0397	0.0233	0.0056	0.0108	0.613	
6	0.0217	0.0091	0.0066	0.0060	0.598	

TABLE II.

Rabbit O. Male. Weight 2.0 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0434	0.0304	0.0011	0.0119	0.838	
2	0.0446	0.0304	0.0026	0.0116	0.833	
3	0.0452	0.0321	0.0024	0.0107	0.793	0.391 gm. thiolactic acid as sodium salt *per os*. (S= 0.1173 gm.)
4	**0.1369**	**0.0960**	**0.0049**	**0.0360**	**0.878**	
5	0.0497	0.0334	0.0033	0.0130	0.847	
6	0.0523	0.0386	0.0044	0.0093	0.865	0.405 gm. thiolactic acid as sodium salt by subcutaneous injection. (S=0.1215 gm.)
7	**0.1547**	**0.0982**	**0.0048**	**0.0517**	**0.915**	
8	0.0328	0.0211	0.0017	0.0100	0.585	
9	0.0529	0.0360	0.0036	0.0133	1.010	

as evidenced by the rise in sulfate sulfur excretion. The changes in the conjugated sulfate sulfur in the feeding experiments are very slight, but it should be noted that similar slight increases were noted in all the other feeding experiments with thiolactic acid not recorded here. The constancy of the nitrogen output

indicates that the changes in the inorganic sulfate are due entirely to oxidation of the sulfur of the thiolactic acid and not to any increased tissue catabolism.

Thioglycollic Acid, $CH_2SH \cdot COOH$.—In contrast to thiolactic acid, thioglycollic acid was found to be somewhat toxic. In the earlier experiments quantities of thioglycollic acid which were comparable to those of the thiolactic acid administered in experiments already discussed were fed. The animals died within a few hours after feeding. The urine in the bladders after death showed a strong nitroprusside reaction.[2] In subsequent studies, in which smaller doses were given, the rabbits exhibited a decided malaise after the administration of the thioglycollic acid. The smaller doses sometimes proved fatal especially when a previous dose had been given (*cf.* Table IV).

In spite of the toxicity of the thioglycollic acid, its sulfur was nearly as completely oxidized and as rapidly eliminated in the organism of the rabbit as was the sulfur of the non-toxic thiolactic acid (Tables III to V). Thus in Table V, 75 per cent of the sulfur of the thioglycollic acid fed was eliminated as "extra" sulfur the 1st day and of this 49 per cent was oxidized to sulfates. In the experiment recorded in Table III, in which a relatively large dose of thioglycollic acid was fed, it would appear that more "extra" sulfur was eliminated than could be accounted for by the compound fed. However, a marked increase in the total nitrogen elimination occurred, presumably occasioned by an increased tissue catabolism due to toxicity of the thioglycollic acid. Normally a fairly constant ratio exists between nitrogen and sulfur in the urine of rabbits. As a result of feeding the relatively toxic thioglycollic acid, a distinct rise in the output of nitrogen occurred. Because of this increased protein catabolism, it is not allowable, in computing the "extra" sulfur of the urine of the experimental days, to subtract the average total sulfur of the preliminary days from the total sulfur of the experimental period, since with the rise in nitrogen there is, presumably, a corresponding rise in the sulfur excretion, sulfur which does not have its

[2] With sodium nitroprusside and ammonia, compounds which contain the mercaptan group give an intense ruby-red color. This test can be applied to urine since creatinine does not give a positive reaction when ammonia is used as the alkali.

TABLE III.

Rabbit P. Male. Weight 2.1 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0496	0.0326	0.0035	0.0135	0.785	
2	0.0407	0.0243	0.0036	0.0128	0.685	
3	0.0389	0.0229	0.0032	0.0128	0.650	0.372 gm. thioglycollic acid as sodium salt *per os.* (S=0.13 gm.)
4	**0.2079**	**0.1129**	**0.0072**	**0.0878**	**1.085**	
5	0.0407	0.0268	0.0051	0.0088	0.995	
6	0.0444	0.0321	0.0035	0.0088	0.833	

TABLE IV.

Rabbit R. Male. Weight 2.1 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0441	0.0280	0.0046	0.0115	1.325	
2	0.0452	0.0262	0.0066	0.0124	1.244	
3	0.0354	0.0190	0.0090	0.0074	1.127	0.296 gm. thioglycollic acid as sodium salt by subcutaneous injection. (S=0.103 gm.)
4	**0.1088**	**0.0545**	**0.0074**	**0.0469**	**1.200**	
5	0.0511	0.0291	0.0069	0.0151	1.146	
6*	0.0305	0.0159	0.0040	0.0106	1.125	

*On the 7th day 0.330 gm. of thioglycollic acid as sodium salt given *per os.* The animal died in 4 hours.

TABLE V.

Rabbit I. Male. Weight 2.3 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0240	0.0103	0.0063	0.0074	0.503	
2	0.0227	0.0091	0.0041	0.0095	0.578	
3	0.0206	0.0062	0.0071	0.0073	0.600	0.205 gm. thioglycollic acid as sodium salt *per os.* (S=0.072 mg.) No albuminuria.
4	**0.0764**	**0.0376**	**0.0031**	**0.0357**	**0.555**	
5	0.0210	0.0091	0.0052	0.0067	0.557	
6	0.0185	0.0055	0.0054	0.0076	0.612	

origin in the sulfur of the compound administered, but which is associated with the increased amount of protein catabolized. In determining the "extra sulfur" in such cases, the value for the normal sulfur excretion should be calculated from the N:S ratio of the preliminary days and the total nitrogen of the experimental day. Thus in the case of Rabbit P (Table III) in which the administration of a relatively large amount of thioglycollic acid resulted in a marked increase in the excretion of total nitrogen, the computation of the "extra sulfur" due to the thioglycollic acid, would be as follows: On the 3 preliminary days the ratio of nitrogen to sulfur was 16.4. Applying this ratio to the experimental day, an elimination of 1.085 gm. of nitrogen would correspond to 0.066 gm. of sulfur. This would give 0.1419 gm. of "extra sulfur" originating from the thio compound fed or a recovery of slightly more than 100 per cent. The "extra" sulfate sulfur (inorganic and conjugated) calculated similarly becomes 0.074 gm. or an oxidation of about 52 per cent of the sulfur recovered in the urine. When one considers that this calculation is only an approximation, the agreement between the amounts of sulfur fed and recovered may be regarded as satisfactory.

Thiodiglycollic Acid, S $(CH_2COOH)_2$.—Tables VI to VIII record the results of feeding and injection experiments with thiodiglycollic acid. This compound produced no toxic symptoms whatever in the dose administered. The total nitrogen output did not vary significantly. Practically all the sulfur fed as thiodiglycollic acid was eliminated as organic sulfur. In five experiments (not all recorded in the tables) in which the acid was administered *per os*, 86, 82, 86, 79, and 83 per cent, respectively, of the sulfur was eliminated as "extra" sulfur in the first 2 days. In three experiments in which it was injected subcutaneously, 93, 100, and 68 per cent was eliminated as "extra" sulfur. No increase in sulfate sulfur excretion could be observed and no evidence of oxidation of the sulfur fed.

The reason for the difference in toxicity between thiolactic and thioglycollic acid is not clear. Both are oxidized to approximately the same extent despite the toxicity of the thioglycollic acid. Lusk (15) has suggested an oxidative desulfurization of cystine similar to oxidative deamination in order to explain the fact that all 3 carbon atoms of cystine are concerned with the

TABLE VI.

Rabbit I. Male. Weight 2.3 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0185	0.0055	0.0054	0.0076	0.612	
2	0.0203	0.0085	0.0044	0.0074	0.580	
3	0.0227	0.0055	0.0096	0.0076	0.548	0.735 gm. thiodiglycollic acid as sodium salt *per os*. (*S* = 0.157 gm.)
4	**0.1580**	**0.0102**	**0.0072**	**0.1406**	**0.538**	
5	0.0247	0.0081	0.0017	0.0149	0.508	
6	0.0233	0.0151	0.0019	0.0063	0.589	

TABLE VII.

Rabbit L. Male. Weight 2.3 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0232	0.0095	0.0044	0.0093	0.515	
2	0.0221	0.0081	0.0037	0.0103	0.550	0.276 gm. thiodiglycollic acid as sodium salt *per os*. (S = 0.059 gm.)
3	0.0234	0.0093	0.0031	0.0110	0.493	
4	**0.0695**	**0.0093**	**0.0040**	**0.0562**	**0.485**	
5	0.0240	0.0060	0.0050	0.0130	0.480	
6	0.0221	0.0060	0.0055	0.0106	0.558	0.251 gm. thiodiglycollic acid as sodium salt by subcutaneous injection. (S = 0.054 gm.)
7	0.0235	0.0140	0.0039	0.0056	0.550	
8	**0.0768**	**0.0150**	**0.0045**	**0.0573**	**0.450**	
9	0.0299	0.0095	0.0064	0.0140		
10	0.0302	0.0182	0.0035	0.0085	0.502	

TABLE VIII.

Rabbit AA. Male. Weight 2.1 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0286	0.0073	0.0095	0.0118	0.515	0.284 gm. thiodiglycollic acid as sodium salt by subcutaneous injection. (S = 0.061 gm.)
2	0.0276	0.0069	0.0104	0.0103	0.573	
3	**0.0689**	**0.0028**	**0.0073**	**0.0588**	**0.408**	
4	0.0203	0.0030	0.0066	0.0107	0.450	

formation of "extra glucose" in the phlorhizinized animal. If such a reaction occurred in the case of the two thio acids under discussion, hydrogen sulfide and either lactic or glycollic acid would be formed. Of these products hydrogen sulfide is rapidly oxidized in amounts far above the lethal dose (16) and lactic and glycollic (17) acids are without toxic effects. The failure of the sulfur of thiodiglycollic acid[3] to be oxidized is in harmony with the theory that only in compounds containing the mercapto group or in compounds which can yield mercapto groups on reduction or hydrolysis is organic sulfur readily oxidized.

SUMMARY.

1. Thiolactic acid (as the sodium salt) when administered to rabbits either subcutaneously or *per os* was readily oxidized, yielding about 50 per cent of the sulfur eliminated in the urine as sulfate sulfur. In the quantities fed, it was entirely non-toxic.

2. The sulfur of thioglycollic acid similarly administered was oxidized only a little less readily than thiolactic acid. It was, however, toxic.

3. No oxidation of the sulfur of thiodiglycollic acid was observed after either oral or subcutaneous administration. No toxic action was noted.

4. It seems probable that of the different types of organic sulfur compounds, only those containing the mercapto group or those which can readily be transformed in the organism into compounds containing this group, are oxidized with ease in the animal organism.

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[3] The results of a feeding experiment with a dog were similar to those recorded here for rabbits. This experiment was carried out by Miss Lucie E. Root under the direction of the senior author (L).

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THE METABOLISM OF SULFUR.

VIII. THE BEHAVIOR OF THIOPHENOL AND THIOCRESOL IN THE ANIMAL ORGANISM.*

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In the preceding paper (1) it has been shown that the sulfur of aliphatic mercapto groups, as in thiolactic and thioglycollic acids, is readily oxidized in the organism of the rabbit, while thiodiglycollic acid, in which sulfur is present as a sulfide, was not oxidized. It was considered probable that in sulfur compounds related to cystine, only the sulfur present as mercapto sulfur or sulfur which could readily be converted to mercapto sulfur in the organism was oxidized to sulfates to any marked degree.

In the present study, we have concerned ourselves with the behavior of mercapto groups attached to the benzene ring as in thiophenol, $C_6H_5 \cdot SH$, and *p*-thiocresol, $CH_3 \cdot C_6H_4 \cdot SH$. Despite the fact that these compounds are rather more toxic than are the aliphatic mercapto derivatives previously discussed and are not absorbed readily, we have obtained evidence which demonstrates that in the organism of the rabbit at least, *no appreciable oxidation of the sulfur of mercapto groups attached directly to the benzene ring occurred.*

EXPERIMENTAL.

The experimental animals, rabbits, and the conduct of the experiments were the same as in our experiments with aliphatic mercapto derivatives (1). The thiophenol and thiocresol were prepared for this work by the organic division of this university.

* An abstract of a thesis submitted by R. M. Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Illinois.

The thiophenol was redistilled and analysis of its sulfur content proved satisfactory. The purity of the *p*-thiocresol was established by its melting point and sulfur determination. Neither of these substances is readily soluble in water. In the earlier experiments, the thiocresol was emulsified by shaking with water to which a few drops of 0.1 N sodium hydroxide and a very little vaseline had been added. This resulted in an emulsion sufficiently permanent to permit administration to animals. Later both the thiocresol and thiophenol were dissolved in olive oil and administered in this form.

TABLE I.

Rabbit G. Male. Weight 1.9 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 5 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0272	0.0150	0.0077	0.0045	0.538	
2	0.0282	0.0124	0.0092	0.0066	0.543	
3	0.0218	0.0112	0.0073	0.0033	0.537	
4	0.0190	0.0071	0.0082	0.0037	0.534	
5	0.0310	0.0056	0.0109	0.0145	0.629	0.235 gm. thiophenol *per os*. (S = 0.068 gm.) Albumin negative. Hemoglobin negative. Pigment negative.
6	0.0243	0.0029	0.0128	0.0086	0.503	
7	0.0155	0.0015	0.0087	0.0053	0.593	

Thiophenol. Thiophenol proved to be toxic when administered to rabbits either orally or subcutaneously. As far as it is possible to judge from the elimination of "extra" sulfur in the urine, absorption did not occur readily either from the intestine or from the tissue into which injection was made. The maximum recovery of "extra" sulfur was noted with Rabbit Y (Table II) in which on the day following the subcutaneous injection of a small amount of thiophenol, approximately 20 per cent of the sulfur injected was eliminated as "extra" organic sulfur in the urine. In other experiments (Tables I to III), oral administration resulted in slight increases in the urinary organic sulfur, increases clearly above the normal fluctuations for this component. No increase in the sul-

TABLE II.

Rabbit Y. Male. Weight 2.8 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0489	0.0251	0.0075	0.0163	0.960	
2	0.0529	0.0265	0.0085	0.0179	0.965	
3	0.0553	0.0294	0.0084	0.0175	0.880	
4	**0.0593**	**0.0213**	**0.0097**	**0.0283**	**0.950**	0.180 gm. thiophenol *per os.* (S = 0.052 gm.) Albumin negative. Hemoglobin negative.
5	0.0437	0.0119	0.0116	0.0202	0.818	
6	0.0409	0.0170	0.0084	0.0155	0.800	
7	**0.0563**	**0.0177**	**0.0094**	**0.0292**	**0.870**	0.187 gm. thiophenol subcutaneously. (S = 0.054 gm.) Albumin negative. Hemoglobin negative. Pigment negative.
8	0.0383	0.0113	0.0066	0.0204	0.855	

TABLE III.

Rabbit U. Male. Weight 2.9 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 10 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0628	0.0375	0.0078	0.0175	1.048	
2	0.0646	0.0390	0.0070	0.0186	1.110	
3	0.0635	0.0354	0.0095	0.0186	1.035	
4	**0.0596**	**0.0284**	**0.0101**	**0.0211**	**0.988**	0.117 gm. thiophenol *per os.* (S = 0.034 gm.) Albumin negative. Pigment negative.
5	0.0687	0.0415	0.0067	0.0205	1.160	
6	0.0667	0.0386	0.0092	0.0189	1.090	
7	**0.0798**	**0.0468**	**0.0140**	**0.0190**	**1.272**	0.130 gm. thiophenol subcutaneously. (S = 0.038 gm.) Albumin negative. Pigment negative.
8	0.0667	0.0203	0.0132	0.0332	1.000	
9	0.0641	0.0212	0.0115	0.0314	1.300	

fate sulfur of the urine was noted with the possible exception of one experiment (Table III) in which a small quantity of thiophenol was injected. A previous oral administration of thiophenol to this animal had failed to increase the elimination of any of the various forms of urinary sulfur determined. The increase in the sulfate sulfur was very slight and was probably within the normal range, specially when it is noted that the total nitrogen was also increased. The increased sulfate sulfur which might be expected to be associated with this increase in nitrogen would account for practically all the increase in sulfate sulfur observed. No albuminuria or hemoglobinuria was observed even in those experiments in which lethal doses of thiophenol were administered.

TABLE IV.

Rabbit A. Male. Weight 1.9 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 5 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0328	0.0233	0.0023	0.0072	0.484	
2	0.0328	0.0233	0.0023	0.0072	0.484	
3	0.0305	0.0234	0.0015	0.0056	0.592	
4	**0.0450**	**0.0219**	**0.0045**	**0.0186**	**0.573**	0.27 gm. thiocresol *per os.* (S = 0.070 gm.) Albumin negative. Hemoglobin negative.
5	0.0306	0.0165	0.0029	0.0112	0.488	
6	0.0220	0.0126	0.0006	0.0088	0.507	

Thiocresol.—In Tables IV to VI are recorded the results of typical experiments in which *p*-thiocresol was fed or injected. Thiocresol produced marked toxic effects, accompanied by a severe albuminuria in some cases. Spectroscopic examination of the urine in a few experiments revealed the presence of oxyhemoglobin.[1] Although there was a marked individual variation in the reaction to thiocresol, in most cases in which albuminuria resulted,

[1] In the tables a few typical experiments only are reported. In all cases, these results were checked with other animals. In order to condense the data these additional experiments are omitted. They are, however, recorded in the original thesis on file in the Library of the University of Illinois.

a dark red pigment (not a blood pigment derivative as shown by spectroscopic examination) appeared in the urine. The presence of this unknown pigment in the urine corresponded roughly to the toxic effect of the thiocresol, but seemed to bear no relation to the

TABLE V.

Rabbit M. Male. Weight 2.3 kilos. Daily diet: 150 cc. of milk and 10 gm. of sucrose.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0288	0.0126	0.0052	0.0110	0.773	
2	0.0284	0.0159	0.0043	0.0082	0.822	
3	0.0198	0.0084	0.0044	0.0070	0.748	
4	0.0286	0.0136	0.0056	0.0094	0.777	
5	**0.0489**	**0.0132**	**0.0052**	**0.0305**	**0.802**	0.3 gm. thiocresol *per os*. (S = 0.078 gm.) Strong albumin. Strong pigment.
6	0.0190	0.0037	0.0092	0.0061	0.407	No food taken.
7	0.0147	0.0036	0.0067	0.0044	0.287	" " " Rabbit very weak.

TABLE VI.

Rabbit E. Male. Weight 2.1 kilos. Daily diet: 150 cc. of milk, 10 gm. of sucrose, and 5 gm. of hay.

Day.	Total sulfur.	Inorganic sulfate sulfur.	Conjugated sulfate sulfur.	Organic sulfur.	Total nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.0253	0.0125	0.0049	0.0079	0.583	
2	0.0244	0.0114	0.0051	0.0079	0.539	
3	0.0327	0.0205	0.0057	0.0065	0.630	
4	**0.0253**	**0.0114**	**0.0038**	**0.0101**	**0.653**	0.355 gm. thiocresol subcutaneously. (S = 0.092 gm.) Albumin negative. Hemoglobin negative. Slight pigment.
5	0.0354	0.0102	0.0055	0.0197	0.780	
6	0.0253	0.0089	0.0062	0.0102	0.786	

hemoglobin since it was present when no test for hemoglobin could be obtained. Attempts to isolate this pigment will be discussed later. There was no evidence of any oxidation of the sulfur of the thiocresol in any of the experiments. The "extra" sulfur recovered

in the organic sulfur fraction in no case corresponded to more than a very small fraction of that administered, but was clearly above the normal variation in every instance.

This failure to recover more than a small amount of the sulfur of the compounds from the urine was apparently due to failure of absorption. After oral administration, the ether extract of the feces gave the orange precipitate with lead acetate which is characteristic of thiocresol. After subcutaneous injection, a hard lump was frequently formed at the site of injection. At autopsy the thiocresol appeared to have crystallized in the tissues at the site of the injection and its presence in ether extracts of such tissues was determined by the lead acetate reaction.

An attempt was made to isolate the urinary pigment which was present after administration of thiocresol. The pigment could be extracted from the urine by ether or chloroform. On evaporation of these extracts, a bright, purple-red material remained. When the urines which contained this pigment were examined spectroscopically no absorption bands could be observed. It was, therefore, not a hemoglobin derivative. The extracted pigment was tested for sulfur by the usual sodium fusion method with negative results. Inasmuch as the amounts obtainable from the urine were so small, no further study of the pigment was made.

It seemed possible that if the mercapto groups of the compounds studied were not oxidized or split from the ring, the organism might protect itself against the toxic thiophenol nucleus by conjugation with sulfuric acid; as is the case with phenol (2). This would give rise to an increase in the conjugated sulfate sulfur of the urine and would result in the presence of conjugated thiosulfates of the type $C_6H_5 \cdot S \cdot SO_2 \cdot OH$. No evidence that the organism availed itself of any such mode of detoxication could be obtained.

SUMMARY.

1. After the administration of *p*-thiocresol and thiophenol to rabbits, there was no evidence that the sulfur of the mercapto groups was oxidized to sulfate sulfur. Despite the insolubility of these thiophenols, evidence of partial absorption was obtained from an increase in the organic sulfur fraction of the urine. This failure of oxidation of the sulfur of mercapto groups attached to the

benzene ring was in marked contrast to the behavior of mercapto groups in aliphatic compounds in which oxidation of the sulfur readily occurred (1).

2. Thiophenol and thiocresol are toxic. After the administration of thiocresol, a red pigment containing no sulfur and probably not a hemoglobin derivative (no absorption spectrum) appeared in the urine.

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Vita.

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